designated C115 and C117, are inherited as gene differences. C115 and mi-3 both have an excess of cytochrome *c* but not as great as in *poky* and both, like *poky*, are deficient in cytochrome *a*. The content of cytochrome *b* is normal in mi-3 and low in C115 but not as low as in *poky*. Cytochromes *a* and *c* have not been detected in C117 but there is an excess of cytochrome *b*. The three strains grow more slowly than wild but faster than *poky*. Strains which carry the mutant genes along with the maternally inherited characters have also been examined. Their properties are much as would be predicted from the characteristics of the individual components.

* This work was supported in part by funds from the Rockefeller Foundation and by funds from the Atomic Energy Commission administered through contract with the Office of Naval Research, U. S. Navy. Contract N6-onr 244. Task Order V.

† Gosney Fellow. Present address: Molteno Institute, Cambridge University, Cambridge, England.

[1] Mitchell, M. B., and Mitchell, H. K., PROC. NATL. ACAD. SCI., **38**, 442 (1952).

[2] Beadle, G. W., and Tatum, E. L., *Am. J. Bot.*, **32**, 678 (1945).

[3] Lein, J., Mitchell, H. K., and Houlahan, M. B., PROC. NATL. ACAD. SCI., **34**, 435 (1948).

[4] Haskins, F. A., Tissieres, A., Mitchell, H. K., and Mitchell, M. B., *J. Biol. Chem.*, **200**, 819 (1953).

[5] Tissieres, A., Mitchell, H. K., and Haskins, F. A. (in press).

[6] Ephrussi, B., and Hottinguer, H., *Cold Spring Harbor Symposia on Quantitive Biology*, **XVI**, 75 (1951).

[7] Ycas, M., and Starr, T. J., *J. Bact.*, **65**, 83 (1953).

[8] Haas, F., Mitchell, M. B., Ames, B. N., and Mitchell, H. K., *Genetics*, **37**, 217 (1951).

[9] Fincham, J. R. S., *J. Biol. Chem.*, **182**, 61 (1950).

[10] Houlahan, M. B., Beadle, G. W., and Calhoun, H. G., *Genetics*, **34**, 493 (1949).

[11] Mitchell, M. B., Pittenger, T. H., and Mitchell, H. K., PROC. NATL. ACAD. SCI., **38**, 570 (1952).

INDUCTION OF CHROMOSOME BREAKAGE AT MEIOSIS BY A MAGNESIUM DEFICIENCY IN TRADESCANTIA

BY DALE STEFFENSEN

DEPARTMENT OF GENETICS, UNIVERSITY OF CALIFORNIA, BERKELEY*

Communicated by R. E. Clausen, April 24, 1953

The mineral elements as constituents of the nucleus undoubtedly participate in important structural and metabolic functions of the nuclear components. Microincineration studies of Scott[1, 3] and Barigozzi[2] showed nuclear minerals, mostly calcium and magnesium, to be closely associated with the chromosomes. Allgén[4] found magnesium to be a constituent of nucleo-histone. Gulick[5] and, more recently, Milovidov[6] have reviewed the problem of inorganic elements within the nucleus. Poulson and Bowen[7] have used radioactive tracer methods in further explorations in this field.

A different method of approach is to test possible effects of alterations in mineral metabolism on the heritable components of the nucleus. Stubbe and Doring[8] found that sulfur, nitrogen, and phosphorus deficiencies increased the "spontaneous" mutation rate in the snapdragon. According to Demerec and Hanson,[9] a high level of manganous chloride considerably increased the mutation rate in *E. coli* (B/r).

The present report concerns the induction of chromosome stickiness by a magnesium deficiency. Because stickiness usually results in chromosome breakage, it is possible in this instance to specify the cytological mechanism responsible for alterations in the heritable material.

Materials and Methods.— Clonal material of *Tradescantia paludosa* (clone 5 of Sax) was grown by culture solution methods of Hoagland and Arnon.[10] Material for cytological examination was fixed in ethanol acetic acid mixtures (3:1) and stained by carmine or Feulgen smear techniques.

Culture Solutions.—In the first experiment, the low magnesium cultures were obtained by adding only limited amounts of magnesium sulfate to solution one.[10] The pH was adjusted weekly to 5.5 with sulfuric acid. The sulfate ion also served as the source of sulfur. For each treatment two sets of four plants each were grown in 7 liters of culture solution. Solutions were changed every two weeks. All material was grown at least three months.

In the second experiment the procedure for a magnesium deficiency was followed,[10] except iron was added as ferric potassium ethylenediamine tetra-acetate.[11] The pH was 5.8 at the start of cultures and before each change, which occurred every six weeks, the pH was no higher than 7.5. For each magnesium level, 10 plants were grown in 40 liters of solution. Most material was not fixed until 4 to 5 months after cultures were started.

Results: Observations on X-ray Control Material.—For the purpose of testing the sensitivity of the chromosomes to ionizing radiation, Tradescantia plants had been grown in culture solutions which were deficient or above normal for several different elements. The results on modification of the x-ray sensitivity will be published elsewhere. Non-irradiated microspores from a low magnesium culture (1.7 p.p.m.) possessed a relatively high percentage of micronuclei. In 422 apparently normal microspores there were 4.9% with one micronucleus and 0.48% with two. Along with the normal microspores there were 396 aborted cells (48.4%) in a total of 818. The micronuclei in the normal cells exhibited extreme variation in size which suggested chromosome breakage as the responsible mechanism. Examination of a limited number of meiotic stages gave evidence of chromosome stickiness. Mitoses in root tips and leaf bases showed neither chromosome aberrations nor sticky chromosomes.

Chromosome Stickiness and Fragmentation at Meiosis: First Meiotic Division.—A second series of low magnesium and magnesium deficient

plants was grown for more detailed analysis. Prophase nuclei were in a tight ball and difficult to spread. At pachytene and diplotene the chromosomes appeared to be somewhat vacuolated. At diplotene and diakinesis a few chromatids and chromosomes were broken. At metaphase I, chromosomes showed an extreme stickiness both within and between bivalents. In a few cases univalents were present but in a frequency of less than 1%. The conventional metaphase I figure, as in material from Hoagland's solution was rare in the magnesium deficient material. Bivalents from the controls were doughnut-shaped and separated normally, while magnesium deficient bivalents were in a tight mass and separated with difficulty. At anaphase I there were bridges and fragments, fragments alone, and chromatin bridges without fragments. The degree of disturbance was quite variable, within cells of the same anther and between anthers of the same bud. Immediately adjacent cells on the same slide had similar frequencies

TABLE 1

	TREATMENT	
	MAGNESIUM DEFICIENCY	CONTROL
No. cells	408	589
Chromosome fragments:		
1/cell	6.4	0.85
2/cell	1.2	0
3/cell	0.25	0
Chromatin bridges and fragments	1.96	0.34
Chromatin bridges (sticky bridges)	3.93	2.04
Attached fragments	0.74	0.17
Lagging chromosomes	0.74	0
Total chromosome fragments	12.2	1.36

NOTE: The frequencies of chromosome aberrations and nuclear disturbance were scored from anaphase II division figures. The table shows per cent of cells with the various aberrations.

of chromosome aberrations as if the effect were nonrandomly grouped.

Second Division of Meiosis.—In the second division the compact prophase and sticky metaphase chromosomes were present but the condition was much less severe than in the first meiotic division. A quantitative evaluation of the chromosome disturbances was possible at anaphase II. The data in table 1 show that the magnesium-deficient cells had a considerably higher frequency of chromosome aberrations than did the cells of the normal Hoagland's solution control. Chromosome fragments, the best estimate of breakage, were nine times more frequent in the magnesium deficient material. It was not always possible to distinguish between a chromatid or chromosome bridge and two unbroken chromosomes forming a chromatin bridge. For this reason all were classified as either chromatin bridges with accompanying fragments and chromatin bridges without fragments.

Tetrad and Early Microspore State.—The first sign of cellular lethality was observed in the very early microspore stage, and little if any cellular death had occurred before this time. Some samples from magnesium-deficient material exhibited 50 to 60% pollen abortion and in a few cases the cells were all aborted or disintegrated. The maintenance of normal cell function by the nucleus in individual microspores is undoubtedly autonomous after the tetrad stage. The cells which died after meiosis probably did so for at least two reasons: first, they did not possess all their essential chromatin, and secondly, the magnesium deficiency *per se* probably had some general adverse effect, such as that responsible for the small necrotic zones in root tips (see later section).

First Microspore Metaphase.—In the few hundred cells examined to date, the chromatid and chromosome breaks at the first mitotic division were less than 1%. Micronuclei were found in 4 to 6% of all cells examined. These mitotic figures and chromosomes were perfectly normal. No chromosome stickiness was present.

TABLE 2

	49 P.P.M. MAGNESIUM (CONTROL)	2 P.P.M. MAGNESIUM	1 P.P.M. MAGNESIUM	MAGNESIUM DEFICIENT
Total no. pollen	8229	5627	7026	6208
Total % of cells with micronuclei	1.03	1.07	1.98	2.00

	COMPARISON BETWEEN 49 AND 2 P.P.M	COMPARISON BETWEEN 1 P.P.M. AND DEFICIENT	COMPARISON OF 49 AND 2 P.P.M. BETWEEN 1 P.P.M. AND DEFICIENT
Chi-square	0.004	0.113	34.45
Probability at 1 D. F.	0.95	0.75	<0.001

Early Binucleate Pollen.—The counting of micronuclei in binucleate pollen is reasonably rapid and reliable for comparisons between different experimental treatments. However, an estimate of the percentage of chromosome fragmentation based on the per cent of micronuclei in the mature pollen grain would undoubtedly lead to a lower value than the real one because of genetic death of cells lacking essential chromatin. Table 2 shows the frequencies of micronuclei in four different levels of magnesium: magnesium deficient, two low-level cultures (1 and 2 p.p.m.), and normal Hoagland's solution control (49 p.p.m.). Eight or more buds from different inflorescences were scored per treatment. The frequencies in the deficient and 1 p.p.m. magnesium pollen were significantly higher ($P < 0.001$) than in the 2 p.p.m. magnesium and control cultures.

Samples from all treatments in both experiments were taken just previous to the onset of severe magnesium deficiency symptoms. The mean frequency of pollen abortion was 2.83% with a range of from 0.8 to 5.5% (12,520 pollen grains scored). With severe deficiency symptoms, the cell

samples which exhibited the highest percentage with micronuclei (5.5 to 6.5%) also had the highest frequency of aborted pollen. A combined count of 1029 microspores gave 50.2% aborted cells. The samples with fewer micronuclei (below 2%) taken from plants most severely effected by the magnesium deficiency had no more than 7.0% pollen abortion. These data then, indicate a positive correlation between the amount of pollen abortion and frequency of micronuclei in magnesium-deficient inflorescences. Such a correlation bears out the contention that an estimate of chromosome breakage produced at meiosis based on the frequency of micronuclei of magnesium-deficient pollen is too low.

Mature Pollen.—In nearly mature binucleate pollen which was severely affected by the magnesium deficiency, a number of bizarre cytological effects were observed. In both experiments a small fraction of the pollen, perhaps 1 to 2%, started precocious division of the generative nucleus. Stages from early prophase (where the chromosomes were visibly split), late prophase, and normal metaphase and anaphase figures were observed. Tri-nucleate pollen grains were the result of such precocious second divisions. In control pollen, either soil or culture solution grown, no such second mitotic division of the generative nucleus has been observed. In normal pollen, the second microspore division takes place in the pollen tube only after the pollen grain has germinated. Occasional mature generative nuclei in magnesium deficient pollen were broken into two or three sections. Other microspores exhibited various degrees of nuclear degeneration. Further study must be done to describe this particular aspect of the magnesium deficiency effect on the nucleus.

Somatic chromosomes from magnesium deficient root tips and leaf bases were examined. Little or no sign of nuclear or chromosome disturbances was found in leaf bases from the magnesium deficient plants. However, there were regions of dead cells in most deficient root tips.

Discussion.—The cytological effects produced by a magnesium deficiency are almost identical to those given by Beadle[12] in the description of the sticky gene in *Zea mays*. In both examples, first and second meiotic divisions are very sticky, and are followed by much degeneration of the microspores while the root tip mitoses appear normal cytologically. The fact that some disturbance of somatic divisions occurred in maize can be detected by the low vigor of the plants and by the presence of streaks of aberrant tissue; with the appropriate genetic factors chimeras could be produced. In addition, chromosome aberrations could be detected in some of the somatic metaphase complements. In the Tradescantia example, the mineral deficiency itself could account for all the observed effects on vigor, and for the presence of small necrotic zones in the root tips. Bands or sectors of aberrant tissue were not observed in the plants and no systematic study of mitotic complements has yet been made. In maize mutations

and chromosome aberrations have been found in the progeny of sticky plants,[13] and seed of the sticky Tradescantia material has been collected for a similar study.

A temperature shock was reported by Sax[14] to produce sticky chromosomes in Tradescantia. Other disturbances such as chromosome aberrations and precocious chromosome development of the generative nucleus were produced as well as asynapsis which in turn gave rise to diploid pollen. The temperature shock treatment caused a precocious division of the generative nucleus. La Cour[15] also found that temperature shock would produce further mitosis in dwarf pollen grains in Tradescantia.

The nuclear effects produced by the magnesium deficiency are in some respects similar to the temperature shock treatments. Just how these two treatments could be related is not clear. The possibility should be considered that a non-specific condition (i.e., starvation) resulting from the deficiency of any essential mineral could cause the meiotic chromosome disturbances produced by the magnesium deficiencies. Some evidence can be presented against such non-specific action of mineral treatments. The frequency of spontaneous chromosome aberrations of material grown in $^1/_{20}$ normal Hoagland's solution (starvation), in iron-deficient or in high-manganese solutions, was no higher than in controls (unpublished data).

As is well known, chromosome stickiness can be brought about by a number of additional treatments and conditions (i.e., light conditions and time of fixation, ionizing radiation, and certain chemicals). Himes[16] using photometric and cytochemical techniques (Feulgen and methyl green) found the sticky chromatin material in *Zea mays* and *Allium cepa* to be desoxyribose nucleic acid (DNA). Whether this DNA was polymerized or depolymerized is still open to question.[17] The sticky chromosome material of the magnesium deficient chromosomes may also be DNA, although this has not as yet been tested.

There is evidence to support the possibility that magnesium is directly concerned with the metabolism of DNA. Stephenson[18] reported that gram positive *Staphylococcus salivarius* when grown on minimal amounts of magnesium or on acid media became gram negative. It is generally conceded that nucleic acid (probably ribose nucleic acid) is the material stained in the gram stain reaction. Tamm and Chargaff[19] investigated the role of magnesium as a cofactor for desoxyribonuclease. Magnesium functioned here both in enzymatic and non-enzymatic break-down of DNA. The possibility that magnesium forms bonds between DNA molecules has been considered.[4] With magnesium present as a constituent of DNA and possibly responsible for the bonding of DNA molecules, the chromosomes would be expected to be more sensitive to breakage in absence of magnesium.

Magnesium, of course, functions as the coenzyme for a great variety of

enzymes in the cell. Of these, some are involved with phosphorylation (i.e., acid phosphatase reportedly inside the nucleus), photosynthesis, decarboxylation, glycolytic pathways (i.e., enolase), and others. Because of the cytological evidence presented, along with the pertinent findings concerning nucleic acid metabolism, the most reasonable hypothesis is that the sticky chromosomes and chromosome aberrations observed are due to the effect of the magnesium deficiency on some aspect of DNA metabolism. However, the possibility that other metabolic functions of magnesium could be responsible for the observed nuclear disturbances cannot be entirely discarded.

The interaction of magnesium with other mineral elements in the cell is extremely complex. For example, *E. coli* (B/r), treated with manganous chloride and then washed with magnesium chloride, had a reduced mutation frequency as compared to the manganous chloride treatment alone.[9] In other micro-organisms Abelson and Aldous[20] found that nickel, cobalt, zinc, and manganese interfered with the normal metabolism of magnesium. Steffensen (unpublished) showed that a high manganese nutrition reduced significantly the number of x-ray induced chromosomal aberrations in Tradescantia. With *E. coli* (B/r), the mutation frequency produced by manganous chloride plus ultra-violet treatment was lower than either one alone.[9] In both irradiation effects, it is not altogether certain whether manganese is involved with a primary effect or a secondary effect of interaction involving magnesium. The highly interrelated nature of mineral metabolism makes experimental interpretation difficult. A great deal of study with mineral elements and their effects on the nucleus must be done before any broad picture can be presented.

Summary.—In Tradescantia, a magnesium deficiency induced chromosome aberrations and stickiness at meiosis but not in mitosis. Chromosome fragments at anaphase II were nine times more frequent in deficient plants than in controls. Chromosome aberrations appearing at meiosis seemed to owe their origin to the stickness.

The frequencies of micronuclei in pollen from plants grown on magnesium deficiencies were increased significantly above controls.

Possible disturbances of DNA metabolism induced by a magnesium deficiency have been discussed.

Acknowledgments.—The author wishes to thank Dr. Spencer W. Brown for suggestions in the course of research and for criticism of the manuscript, and Drs. A. H. Sparrow and Everett R. Dempster for reading the manuscript. Dr. A. E. Brandt did the chi-square test.

* Present address: Biology Department, Brookhaven National Laboratory, Upton, Long Island, N. Y.

[1] Scott, G. H., *Bull. Histol. Appl. Physiol. et Path.*, **7**, 251–256 (1930).

[2] Barigozzi, C., *Ibid.*, **15**, 213–219 (1938).

[3] Scott, G. H., *Proc. Soc. Exptl. Biol. and Med.*, **45**, 30–31 (1940).
[4] Allgén, L. G., *Acta Physiol. Scand.*, **22**; Suppl., **76**, 140 pp. (1950).
[5] Gulick, A., *Bot. Rev.*, **7**, 433–457 (1941).
[6] Milovidov, P. F., *Protoplasma Monographien*, **20**, Erster Tell (1949).
[7] Poulson, D. F., and V. T. Bowen, *Exptl. Cell Research, Suppl.*, **2**, 161–180 (1952).
[8] Stubbe, H., and Doring, H., *Z. indukt. Abstamm. u. Vererb. Lehre*, **75**, 341–351 (1938).
[9] Demerec, M., and Hanson, J., *Cold Spring Harbor Symp. Quant. Biol.*, **16**, 215–228 (1951).
[10] Hoagland, D. R., and Arnon, D. I., *Calif. Agric. Exptl. Sta. Cir.*, 347, 32 pp. (1950).
[11] Jacobson, L., *Plant Physiol.*, **26**, 411–413 (1951).
[12] Beadle, G. W., *Z. indukt. Abstamm. u. Vererb. Lehre*, **63**, 195–217 (1932).
[13] Beadle, G. W., *Cytologia*, Fujii Jubilee Volume, 43–56 (1937).
[14] Sax, K., *Am. J. Bot.*, **24**, 218–225 (1937).
[15] La Cour, L. F., *Heredity*, **3**, 319–388 (1949).
[16] Himes, M. H., *Genetics*, **35**, 670 (1950).
[17] Alfert, M., *Biol. Bull.*, **103**, 145–156 (1952).
[18] Stephenson, M., *Bacterial Metabolism*, 3rd ed., Longmans, Green and Co., London, 1949.
[19] Tamm, C., and Chargaff, E., *Nature*, **168**, 916 (1951).
[20] Abelson, P. H., and Aldous, E., *J. Bact.*, **60**, 401–314 (1950).

---

*UNITY OF THE VEGETATIVE POOL IN PHAGE-INFECTED BACTERIA**

BY N. VISCONTI AND A. GAREN

CARNEGIE INSTITUTION OF WASHINGTON, DEPARTMENT OF GENETICS, COLD SPRING HARBOR, N. Y.

Communicated by M. Demerec, May 4, 1953

A considerable body of genetic[1–12] and chemical[13–18] facts has led to the following interpretation of the growth-cycle of T2 phage. Upon infecting a bacterial cell a phage particle is transformed into a noninfective intracellular form called *vegetative* phage. This vegetative form can multiply and, if a bacterium has been mixedly infected with two or more related phages which differ in their genetic composition, can also undergo genetic recombination. During the first few minutes after infection, called the *eclipse period*, only vegetative particles are present within the bacterium. Afterward infective (or *mature*) particles begin to form and accumulate at a constant linear rate until the bacterium lyses, at which time several hundred mature phage progeny are liberated. The vegetative particles are pictured as forming an intracellular *pool* from which units are removed at random and transformed irreversibly into mature phage particles, which then remain inert with respect to the multiplication and recombination processes of the vegetative particles. Thus the three critical processes which